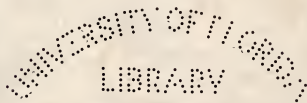


THE EFFECT OF REDUCED PRESSURE
ON THE EXTRACTION OF DRUGS

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I. INTRODUCTION

In the year 1816 P. F. Real constructed a pressure apparatus for the extraction of drugs. Since that time many types of apparatus have been constructed using pressure or reduced pressure or both with the view in mind of preparing better fluidextracts, tinctures or dry extracts. Several investigators have reported that a variance in pressure increased the extraction of the particular drug on which they were experimenting. However it may also be said that, while there have been some investigators who favored the use of increased or decreased pressure, there have been others who did not favor the use of these methods in the extraction of drugs. Even at the present time there still seems to be some doubt as to the effect of pressure or reduced pressure in the extraction of drugs.

The purpose of the present investigation was to clarify the information previously published and to conduct experimental work with the hope of drawing more definite conclusions as to the effect of reduced pressure on the extraction of belladonna root and yellow cinchona.

II. HISTORICAL REVIEW

1. Use of Pressure and Reduced Pressure in Drug Extraction from 1816 to 1835. For over a century there has been some discussion and experimentation on the use of pressure and reduced pressure in percolation. Many forms of apparatus have been devised for applying extra pressure or reducing the pressure. Of the many types of apparatus introduced, the first made use of hydrostatic pressure of the liquid. Other types made use of air pressure applied on the surface of the menstruum above the drug

by direct application of air pressure on the surface of the liquid or by evacuating the receiver, while in some cases mechanical pressure was used on the drug. The different types of apparatus will be discussed by classes in the paragraphs which follow.

A. Hydrostatic Pressure of the Liquid. The first apparatus of this kind was constructed by P. F. Real and described by C. L. Cadet (1). Real's press consisted principally of a receptacle in which was placed the vegetable powder to be extracted. A long pipe connected to the top cover of the receptacle extended straight up for any desired distance, frequently fifty to sixty feet. The hydrostatic pressure exerted by the column of water was supposed to increase the percentage of extractive in the first percolate.

Real soon realized that a high water column was not convenient. He then devised an apparatus (1) in which a column of mercury was used to produce the hydrostatic pressure in place of the water. This reduced the length of the column, because a short tube of mercury produced the same pressure as a long tube of water, due to the greater specific gravity of mercury. Real's press, which utilized mercury pressure, consisted of two containers, one for the mercury and the other for the powder to be extracted. The two containers were connected by an iron tube in the form of an arc. At the top of the arc was placed a funnel with a stopcock. The powder to be extracted was placed in the drug container and the mercury in the mercury container. Water was poured in the funnel at the top of the arc until the arc tube and the reservoir above the drug were filled. The vertical tube extending from the mercury container was filled with mercury.

The mercury exerted a pressure on the water, which passed through the powder and fell into a receptacle placed under the drug container.

Geiger devised two modifications of the Real press. His modifications worked on the same principle as the Real press, with the exception that the pressure column was filled by means of a siphon. In Geiger's first modification (2) the drug was placed in an extraction flask between two perforated plates. The pressure column was fastened on top of the extraction flask and extended upward. The upper end of the column was bent so that it could be immersed in a vessel of water, thereby obtaining a siphon effect. The bottom of the extracting flask was open so that the percolate could flow out. The entire apparatus was set upon a tripod.

Geiger's second modification (3) was similar to his first apparatus. However in the second modification, Geiger placed about one-fourth of the extraction flask in a second cylinder, thus leaving a space below the bottom of the extraction flask for receiving the percolate.

Dobereiner (4) devised a modification of the Real press in which mercury was used to create pressure on the menstruum. This apparatus consisted of two chambers, one for the drug to be extracted and the other, which was placed above the extraction chamber, for the menstruum. A vertical glass tube about four feet in length was connected to the top of the menstruum chamber. The two chambers were connected by a short tube. After having placed the drug in the extraction chamber and the menstruum in the menstruum chamber, the long tube was filled with mercury, thus increasing the hydrostatic pressure. As percolation proceeded the mercury gradu-

ally rose in the menstruum chamber. The mercury was not allowed to reach the upper end of the short tube, connecting the two chambers, or it would have flown into the extraction chamber.

Brandes (5) designed an apparatus very much on the order of the Real press. It consisted of a wooden vessel with holes in the bottom. The holes were stoppered with corks. On the cover of the vessel was a vertical tube twenty feet in length. The drug was placed in the wooden vessel and covered with a perforated plate. The cover with the vertical tube was attached and water poured into the tube. After the corks had been removed the percolate flowed from the wooden vessel through the holes in the bottom.

Wurser (6) said that the pressure exerted in the original Real press was not utilized. His apparatus differed from the Real press in that a stopcock was attached to the bottom of the extraction vessel so as to regulate the discharge. A Senguerd stopcock was also placed just above the extraction vessel on the pressure column. By the use of the two stopcocks the pressure could be regulated. By use of the Senguerd stopcock the liquid remaining in the pressure column could be withdrawn without coming in contact with the exhausted drug.

Beindorf's modification of the Real press as described by P. L. Geiger (3) consisted of two compartments, one for the drug and the other for the liquid. The upper compartment which contained the liquid was attached to a high, vertical column. The drug in the lower compartment had perforated plates placed above and below. The whole apparatus was placed on a table which was constructed so that the apparatus could be

tilted in order that the liquid above the drug could be drawn off through a stopcock at the top of the liquid compartment.

B. Air Pressure Applied Directly on the Surface of the Menstruum Above the Drug. Semmelbauer (7) was one of the first to break away from the principle of the Real press which obtained pressure by means of a long, vertical tube filled with a liquid. Semmelbauer used an air compression machine.

Schubart designed an apparatus, which was described by H. Wurzer (8), in which the pressure was produced by aid of a pressure pump. The process used consisted in pouring warm or hot water on the substance to be extracted and applying pressure by means of a hand lever arrangement. The drug was placed in a copper vessel and a tin perforated plate placed below and above. However if the drug was placed in a flannel bag the top perforated plate was not necessary. The pressure was applied as long as desired, then the stopcock under the copper vessel was opened and the solution flowed out.

Payen (9) constructed an apparatus in which the desired pressure was obtained by means of a small pressure pump. The extraction chamber was a glass cylinder open at both ends and was attached in a vertical position to a receiving flask by the use of a perforated cork. Filter paper or linen was tied over the lower end of the extraction cylinder, the drug placed inside the cylinder and the air pump fastened on the top of the extraction cylinder. A manometer was placed on the connection between the pump and the extraction cylinder, so that the pressure could be measured.

Romershausen (10) designed an apparatus in which compressed steam forced hot water through the drug mass to be extracted. In this modification Romershausen used two containers to heat the water. In the first container, which was open at the top, water was heated and, by means of a stopcock, was then run into the second container. The stopcock was closed and the water further heated, thus compressing the steam. The stopcock between the second container and the extraction vessel was opened, and the compressed steam forced the hot water up through the drug which was in the extraction vessel. The solution was drawn off through an opening which was placed just above the drug mass.

Beral's apparatus (11) worked on the same principle as Payen's apparatus which was discussed above. Beral, however, used a tin extraction vessel instead of a glass cylinder as used by Payen. Beral did not describe any manometer attachment as was used in Payen's apparatus.

C. Air Pressure Applied By Evacuating the Receiver. In 1818 Kastner (12) used a vacuum pump for the extraction process. Since that time several types of apparatus have been constructed using atmospheric pressure by withdrawing the air from the receiver.

Romershausen (10) invented an air press apparatus for the extraction of drugs. The exhaust pump was connected to the bottom of the extraction vessel by means of a pipe. The drug was placed in the extraction vessel and the menstruum added through the top of the vessel. On working the pump a vacuum was formed and the atmospheric pressure pushed the menstruum through the drug, through the pump and out through an opening

at the top of the pump into a vessel.

Beindorf (3) modified Romershausen's apparatus (10) by making the receiver in the shape of a half cylinder. The half cylinder was placed horizontally on a stand and allowed to rest on its flat side. The vacuum pump was placed on top at one end of the receiver and the extraction chamber was screwed on the other end of the half cylinder. The menstruum was added through the top of the extraction chamber. By the use of the pump a vacuum was formed in the receiver.

Another of Beindorf's modifications of Romershausen's apparatus operated similarly to the one just described. In this second modification the receiving vessel was globe shaped. The vacuum pump as used above was connected to the receiving vessel by means of a pipe. A long tube, as in the Real press, was placed above the extraction chamber thereby providing more pressure. The tube was fed from a vat of the solvent by a siphon arrangement.

Martenstein (13) devised an apparatus in which he made use of a partial vacuum. A tall cylinder, which was to contain the drug, was placed on a large receptacle. The receptacle was filled with water, placed over a fire and the water evaporated. The drug was packed in the cylinder and the menstruum added through the top of the cylinder. The apparatus was then placed in water and the cooling effect produced a partial vacuum in the receptacle.

Beral (14) modified his apparatus by applying vacuum in the receiver by means of a vacuum pump. This apparatus was similar in construction to his apparatus in which air pressure was applied on the menstruum.

D. Mechanical Pressure Applied to the Drug. Hæmle (15) used an apparatus in which mechanical pressure was applied to the drug. The drug was placed in a cylinder with a perforated bottom. An inner cylinder, also having a perforated bottom, was slipped in the drug cylinder and pressed on the drug. This inner cylinder was tied in place by means of strings, which were then moistened, thus producing pressure on the drug. The menstruum was then applied through the top of the inner cylinder and allowed to percolate.

2. Use of Pressure and Reduced Pressure in Drug Extraction from 1835 to 1938. Of the various types of apparatus using pressure or reduced pressure in the extraction of drugs during this period there were some that still utilized hydrostatic pressure. Other forms of apparatus were devised so that both hydrostatic pressure on the liquid and mechanical pressure on the drug could be used. Of the many forms of apparatus devised most of them used air pressure on the surface of the liquid.

Many types of apparatus were constructed so that the receiver, percolator or macerating vessel could be evacuated. There were many types of apparatus which used air pressure on the surface of the liquid and at the same time evacuated the receiver. There were still other forms of apparatus which used mechanical pressure applied to the menstruum or to the drug. The different types of apparatus will be discussed by classes in the paragraphs which follow.

A. Hydrostatic Pressure on the Liquid. In Christison's Dispensatory (16) a pressure percolator was described that used mercury to obtain the desired hydrostatic pressure. The percolator was cylindrical and was

covered with calico at the lower end. The percolator was then connected to a receiver by means of a cork and a tube connected the top of the percolator to the menstruum chamber. A vertical tube was attached to the menstruum chamber and mercury was poured in the vertical tube.

To this group of percolators, which like the Real filter press used hydrostatic pressure, belonged the Loysel percolator which was constructed in 1854 (17). This apparatus consisted of a Real press with several sieve bottoms standing above each other in the drug chamber.

Proctor (18) suggested, in addition to the usual cylindrical tube and receiver, a cylinder of tin plate or other suitable material, closed at both ends, and fitted loosely within the percolation tube. The cylinder floated in the liquid. The object was to get a slightly increased hydrostatic pressure with a small quantity of the solvent.

B. Hydrostatic Pressure on the Liquid and Mechanical Pressure on the Drug. Rosenwasser (19) used a process of percolation in which hydrostatic pressure and mechanical pressure were used. He simply reversed the ordinary percolator, attaching a long rubber tube to the nozzle and connecting it to the menstruum reservoir. First the percolator was placed upright and a porous diaphragm was placed in position at the bottom of the percolator. The drug was, after previous moistening, packed tightly in the percolator. A porous diaphragm was placed on the drug and fastened to the body of the percolator. A plate was secured to the top of the percolator. The percolator was then reversed and attached to the menstruum reservoir. In this manner the drug was held in place on all sides, so that it could not expand beyond its original limits. The drug swelled

considerably in some instances; thus the greatest possible compactness was secured. As the height of the column of liquid increased the pressure and velocity of the liquid increased. This arrangement produced a force that could compete with lateral pressure, no matter how much this would increase by the subsequent swelling of the drug. Instead of having to unpack the percolator and repack it more loosely, it was only necessary to increase the height of the tube and reservoir.

Berry introduced a percolator which did not differ from Rosenwasser's percolator except perhaps in the material used and the manner of fastening the diaphragms (20).

It is interesting to note that Rosenwasser said that Berry had infringed upon his patent of a pressure percolator. The case was carried to court and the decision of the court (21) was as follows: "The suit of Nathan Rosenwasser et Al. v. John Berry for an alleged infringement of Rosenwasser's patent (No. 256,504) for a pressure percolator, has been recently dismissed by the Circuit Court of the United States, for the District of Maine, the complainant not having established the fact that Berry's percolator was in reality an infringement".

Hallberg (22) used a cylindrical glass percolator in which the drug was placed upon a diaphragm resting in the neck of the percolator. The superior diaphragm was placed upon the drug and pressed down firmly by means of an affixed rod. An air-tight cover was fastened to the top of the percolator by means of screw clamps, a central hollow piston was forced down over the rod of the diaphragm provided with a spiral screw, and a safe degree of compression of the contents effected. The reservoir

was connected to the top of the percolator by means of a tube. The reservoir could be raised about ten or fifteen feet.

Cohn (23) used a can of any suitable size for the percolator. Two oblong holes were cut at opposite sides about one inch from the lower edge of the can and a block run through the oblong holes. A round wooden block, with a hole cut in the center, was made to fit inside the percolator. The drug was moistened, and, after swelling, was packed firmly in the percolator. The circular block was inserted and forced tightly against the drug. A tube was connected to the hole in the circular block and connected to the receiving flask. A reservoir of menstruum was placed above the percolator and was connected to the percolator by means of a tube attached to the spout of a small funnel which had been soldered on the top of the percolator. Cohn stated that the pressure obtainable was quite astonishing and was directly proportional to the height of the reservoir.

C. Air Pressure Applied Directly on the Surface of the Menstruum

Above the Drug. In percolation under strong pressure Signoret (24) used compressed air upon the surface of the liquid in the percolator. The apparatus consisted of a reservoir of metal for the compressed air, of such strength as to resist from two to eight atmospheres, according to the force desired. Attached to this reservoir was a forcing pump which worked by a lever. On four opposite points in the sides of the reservoir were placed four cocks with coupling screws for attaching tubes connecting the reservoir with the tops of four percolators. In employing this apparatus, the powdered material, perhaps previously moistened, was

placed in the percolator and the menstruum poured upon it. The pressure of the reservoir was then applied. Signoret believed that by this arrangement, substances were more thoroughly and quickly exhausted than by maceration or ordinary displacement. Wine of cinchona was made in twenty minutes.

Whitebread (25) described a regulating percolator invented by George McPherson, Chicago, Illinois, and patented April 27, 1875. This invention consisted of a faucet connected to a closed cap upon the head of the percolator, the faucet being provided with depressions upon its outer periphery, whereby the operator was enabled to control the admission of air, to regulate the progress of the menstruum through the drug in the percolator and to secure, in a convenient manner, time for the more thorough extraction by maceration of the medicinal principles contained in the drug, while, during the process, loss of alcohol, ether or other volatile and expensive liquids by evaporation was entirely prevented. The invention further consisted of a pump or air-forcing apparatus, whereby the air could be compressed above the menstruum and the percolation accelerated according to the degree of pressure.

Hinsdale (26) designed a pressure percolator. He claimed that the operation of percolation was hastened by the use of compressed air. He used a cylindrical chamber in which was placed the substance to be percolated. The upper end of this chamber was attachable to an air chamber; the connection was made air-tight by means of screw threads and rubber gaskets. A rubber bulb provided with valves was attached to the air chamber by a flexible pipe. With the aid of the rubber bulb the tension

of the air in the chamber could be increased.

Phillips (27) designed a pressure percolator in which the compressed air was obtained from the hydrostatic pressure of the menstruum. A bottle was selected which would be filled only two-thirds full by the drug. The moistened drug was placed in the bottle which was then stoppered. The stopper had two holes in which glass tubes were placed. One of the glass tubes was just long enough to go through the stopper, the other almost reached the bottom of the bottle. The ends of the glass tubes in the bottle were covered with muslin. After the bottle had been stoppered it was inverted and the drug packed as firmly as possible by shaking and jarring the bottle against the hand. The long glass tube was connected to a rubber tube which at the other end contained a funnel or vessel in which the menstruum was added.

Hoseason (28) described an extraction apparatus which was constructed to permit its use for a variety of purposes. It consisted essentially of a jacketed copper percolator, tinned internally, and of a tubular condenser. The lid of the percolator was fitted air tight. The tubular condenser was attached on the top of the percolator. A sufficiency of menstruum was added and maceration was allowed to proceed for twenty-four hours. The first percolate could then be obtained by applying the force of an air pump where the condenser was connected.

Extraction of vegetable drugs under pressure with carbonic acid was described (29). Into the menstruum surrounding the drug carbon dioxide was passed under pressure. If necessary the mixture was heated. Then the pressure was rapidly removed. The carbonic acid, which had diffused

into the drug, ruptured the tissues so that extraction was complete.

The process was patented by H. Deininger of Berlin.

Nunn (30) recommended his apparatus for percolation under pressure and stated that it could easily be constructed with parts of apparatus commonly employed in laboratories. The substance to be extracted was placed in the percolator, the menstruum was added and after sufficient maceration the stopcocks were opened and pressure applied by means of a pump such as was used for inflating bicycle tires.

Referring to Nunn's method of percolation under pressure Cowley (31) observed that the fact that the pressure was intermittent was a serious disadvantage. Cowley did not advocate the use of pressure in percolation but stated that constant pressure doubtless was of advantage in hastening percolation in some cases such as for mucilaginous or finely powdered drugs. The plan adopted consisted in displacing air from a bottle by allowing water to run into it from a reservoir placed above the bottle. The displaced air was conducted through a tube into the space above the menstruum in the percolator. The supply of water could be obtained from the tap of the regular water supply, or the pressure could be increased or diminished by raising or lowering the reservoir. Preliminary maceration or intercepted maceration could be obtained at will by lowering the water reservoir to the level of the percolator.

J. Herzog (32) introduced an apparatus in which the air above the menstruum was compressed by a pressure pump.

W. Lenz (33) described a new vapor and pressure percolator in which the substance to be extracted was placed in a copper percolator surround-

ed with a jacket. The menstruum was added and steam was passed around the percolator thus heating the menstruum. By the aid of a bicycle pump, which was connected to the cover of the percolator, a pressure of two or three atmospheres could be produced.

D. By Evacuating the Receiver, Percolator or Macerating Vessel. For cold displacement Vielguth and Mentwich (34) constructed a suction pump, which withdrew the air from the receiving vessel, so that the atmospheric pressure forced the liquid in the percolator through the finely powdered substance.

The use of vacuum to increase penetration of the menstruum into the drug was suggested by Duffield. In a letter to William Procter, Jr., (35) Duffield stated that more perfect maceration could be obtained if the ground drug was placed in a strong cylinder, the air pumped out and the requisite amount of menstruum admitted. He stated that the pores of the comminuted drug gave up the air enclosed in them and when the menstruum was allowed to flow in it was forced into the pores by pressure of the air outside. Duffield stated in his letter that he macerated in vacuo for six to ten days.

Fairthorne (36) described a displacement apparatus in which the drug was moistened and packed in the extraction chamber and air withdrawn from the chamber by means of an air pump. The menstruum was then added and the materials allowed to macerate for several days. In order to start percolation, the receiver was exhausted of air and the stopcock between the receiver and extraction chamber was opened. The saturated fluid began dropping and continued to do so, as long as the force of the vacuum in

the receiver was equal to or greater than that in the upper vessel. When the flow of liquid diminished, air was admitted above the drug. The air was drawn through the drug and carried with it much of the remaining liquid. To finish the operation air was forced into the percolator by means of the pump. In using this apparatus for preparing fluidextracts, Fairthorne stated that only one pint of menstruum for every sixteen ounces of the material was employed.

Maben (37) described the apparatus which had been successfully used by him to hasten the process of percolation. It consisted of a water vacuum pump, which was connected to the receiving flask, and an ordinary York Glass Company percolator.

Platt (38) devised a pressure percolator which was operated on the exhaust principle of an aspirator. The cork of the receiving vessel was provided with glass tubes, one connected to the percolator and the other with an aspirator. The latter was merely a "T" tube connecting the hydrant with the glass tube.

Hostelley (39) described an apparatus used for filtration or percolation under pressure. He designed two forms of apparatus. In the first of these, pressure was produced by suction from a vacuum pump which was connected to the receiving flask. The liquid to be filtered or the menstruum for the percolation was supplied by the aid of a siphon from a suitable elevated reservoir which was capable of being raised or lowered. In the second form of apparatus, the vacuum pump was combined with the apparatus itself and consisted of an inverted one-gallon bottle of water, which, as the contents flowed from it, aspirated at the expense

of the air in the receiving bottle.

Rogers (40) stated that he used an extraction apparatus with which excellent results were obtained in the analysis of tea, coffee, etc. where an aqueous infusion was necessary. The apparatus consisted of three flasks or chambers, one for the menstruum, one for the material to be extracted and one for the percolate. A filter pump was connected to the percolate flask and suction applied, thus causing the liquid to pass through the material in the extraction flask. The apparatus was so arranged by means of tubes that the percolate could be returned to the menstruum flask and again run through the material to be extracted.

Beard (41) described a continuous percolation process under reduced pressure. The process worked on the same principle as the Soxhlet extractor. Heat and suction were carefully regulated so that the menstruum would boil between 35 and 40° C. Beard stated that he believed the advantages of the process were the saving of menstruum and economy of time.

Hambleton (42) and Macheboeuf and Fethke (43) also used the Soxhlet principle under reduced pressure for the extraction of materials.

In 1934 the "Mulcolator-Kessler" apparatus (44) and the process of making fluidextracts by mulcolation were described. The apparatus consisted of six thick-walled tubes, each about 1 meter long and 6 cm. wide, connected end to end and terminated by a suction flask attached to a water pump. The apparatus was intended to make 2400 Gm. of fluid-

extract from 2400 Gm. of drug. The drug was divided into three equal portions and after moistening the first 800 Gm. of drug with 400 Gm. of menstruum and macerating for 12 hours, it was packed in tubes one and two. Menstruum was then run into the tube at the rate of 20 to 25 drops a minute, and 400 Gm. of percolate was collected in the suction flask attached at the end of tube two. The 400 Gm. of percolate collected was used to moisten the next 800 Gm. of drug, while from tubes three and four 200 Gm. and 400 Gm. respectively of reserve were collected. Another 400 Gm. portion of percolate was collected from tube four for moistening the next 800 Gm. of drug and from tube five 400 Gm. and tube six 1400 Gm. of percolate were reserved. The 200 Gm. from tube three, 400 Gm. from tubes four and five, along with 1400 Gm. from tube six made a total of 2400 Gm. finished fluidextract from 2400 Gm. of drug. The menstruum was displaced from the extracted drug by water.

In 1935 E. Kessler (45) described his process of preparing a fluidextract of condurango as follows: Two hundred and fifty grams of dry moderately coarsely powdered condurango was packed in a tube 1 meter long and 28 mm. wide. The drug filled the tube 95 cm. in height. The menstruum was supplied at the top of the tube by a vessel provided with a device for adjusting the delivery. A suction flask was attached to the tube and the apparatus was evacuated by means of a water pump. The menstruum was allowed to flow in the tube on the drug at the rate of two drops a minute until the drug was entirely penetrated by it. The valve between the water pump and the suction flask was then closed and the percolation was carried out at the rate of

about two drops a minute. When percolation ceased water was let in from the supply vessel at the same rate until the menstruum was entirely displaced from the drug. In order to collect 250 Gm. of percolate about 36 hours was required.

Schmierer (46) described an apparatus for extracting vitamin containing juices from fruits and vegetables in a vacuum.

Husa and Yates (47) macerated belladonna root in No. 40 powder in vacuum preceding percolation. A flask containing the drug was evacuated for three hours. Sixty cubic centimeters of the official menstruum was then added through a separatory funnel placed through the stopper, the flask being shaken until the powder appeared uniformly damp. The mixture was macerated for six hours. The drug was then packed and percolated immediately.

C. J. Blok and H. J. A. Ter Wee (48) employed a somewhat similar apparatus devised by Kessler (45). The drug was packed in a closed cylindrical tube, the lower end of which was connected with a suction flask. When the suction flask was evacuated the liquid was forced through the drug by the air pressure above. The rate of flow was adjusted by means of a pinch-clamp placed between the reservoir and the cylindrical tube. This process of extraction is known as evacuation.

W. H. Jackson invented a self-contained vacuum system, as described by Chilson (49) which shortened the time required for the extraction of vanilla beans. This method consisted in grinding the beans under vacuum in the menstruum, reducing them to a sludge which was then pumped through a filter press. The unit consisted of a specially de-

signed tank containing a stellite knife propellor which rotated at a speed of several thousand revolutions per minute. The beans were reduced to a size equivalent to that usually used in ordinary extraction methods. With this method the beans were chopped to about a number eight mesh in ten minutes. The mixing was continued for about five hours and the particles of vanilla beans were reduced until they were so small that the batch looked like soup. Samples of extract examined after twenty minutes of mixing showed a strength equivalent to that obtained formerly after several days of maceration.

For laboratory extraction Chilson (50) suggested using a new washing machine as the extractor. To operate the unit you would place the drug in the mixing tank, add sufficient solvent, bolt on the cover and mix for a few minutes. Then you would produce a vacuum on it, hold it for about fifteen minutes, and then break it by opening the vent cock on the cover. This will impregnate the drug thoroughly.

F. Chilson (51) described the Stokes Vacuum Process and the Scott Process for the extraction of crude drugs. The Stokes Vacuum Process was installed by Chilson at the Grove Laboratories. As installed in the Grove plant the apparatus consisted of a steam jacketed vacuum extractor and dreg still, a vacuum pan with breaker agitator and the necessary vacuum pumps, condensers and percolate tanks. A charge of drug ground to about a twelve mesh was put into the extractor which was then tightly sealed. A high vacuum was drawn on the machine and the alcohol sucked into it. The vacuum was broken to impregnate the drug and the lot was mixed for a variable period of time. Then the perco-

late was run or pumped off into collection tanks and successive washes run in the same way. Meanwhile the percolate was drawn into the vacuum still and concentrated to a dry powder.

The Scott Process is similar in construction and operation to the Stokes apparatus. Both operate under vacuum, both mix the drug, both are equipped with filters through which the percolate is run off. In both processes the solvent can be recovered from the exhausted marc before it is dumped from the extractor. The only element of difference consists in the fact that the Scott equipment discharges the marc through the head of the cylinder and the Stokes unit through the bottom.

W. C. Peck (52) described a vacuum agitating extractor. The plant was operated by charging the drug into a jacketed cylindrical extraction vessel fitted with an agitator. The extraction vessel was then evacuated and the menstruum allowed to flow on the evacuated drug. The agitator was run for a short time and the mass thoroughly mixed under vacuum. The air was then allowed to re-enter the vessel and the percolate run off to receiving tanks. A series of washes was run through the extraction vessel in this manner.

E. Direct Application of Air Pressure on the Surface of the Liquid and Evacuating the Receiver. A percolator invented by Albert Merrell, Cincinnati, Ohio, and patented June 14, 1870 was described by Whitebread (53). This apparatus consisted of two conical-shaped vessels one resting above the other in a suitable frame. In using the apparatus, the substance to be percolated was placed in the upper

vessel on a perforated septum. The covers were tightly bolted on, and the upper, middle and lower openings closed. The air was then exhausted from the entire apparatus. By opening a valve at the top of the upper vessel, the percolating liquid was allowed to flow into the vessel. After maceration had progressed for a proper length of time, the valve between the two vessels was opened and the percolate passed into the lower vessel, the receiver. The operation could be hastened by pumping air into the upper vessel through a valve provided for that purpose.

Thomson (54) described a process with apparatus for manufacturing fluidextracts on a large scale. The apparatus consisted of three chambers. The upper chamber constituted the reservoir in which the menstruum was placed and subjected to a pressure of three or four atmospheres. The middle vessel was the percolator in which the properly moistened material was packed between two diaphragms and macerated with the menstruum in a partial vacuum. The lower vessel was the receiver, in which a vacuum was produced before the apparatus was put into operation. After sufficient maceration of the drug, communication between the three chambers was opened. Percolation immediately began and the liquid was forced through the drug under considerable pressure.

Marpmann (55) recommended an apparatus for the extraction of vegetable as well as animal substances in the preparation of fluidextracts. Its construction was such that the extraction could be conducted under pressure in an atmosphere of carbon dioxide or nitrogen, whereby putrescence of the material was prevented. The pressure-gas was con-

nected to the drug chamber which was provided with a revolving shaft consisting of horizontal spokes and a scraper near the bottom. At the bottom of the extraction chamber was placed a wire sieve and asbestos filter. As extraction was produced the horizontal spokes kept the contents of the percolator in continuous motion, the sieve meshes being kept open by the scraper. The suction pump was connected to the receiving vessel.

Fluidextract of cinchona was prepared by diacolution by H. Breddin (56). A battery of nine tubes, each 80 cm. long and 1.7 to 1.8 cm. wide was used. Six hundred grams of cinchona was moistened, macerated and packed in the tubes. By use of the drip chamber the menstruum was allowed to enter the system at the rate of one and one-half drops a minute and was forced through the drug by a pressure not exceeding one and one-half atmospheres. The receiver was evacuated to help the menstruum penetrate the drug and to help establish the correct rate of flow. The process of making 600 Gm. of fluidextract from 600 Gm. of drug lasted about 20 days.

F. Mechanical Pressure Applied to the Menstruum. Flowers (57) used a percolator which consisted of a metal cylinder or tube with an air tight piston. The rod of the piston was threaded so that the piston could be screwed down tightly upon the menstruum. Flowers presented three tinctures prepared by the above process, however the samples submitted were not analyzed to see if they measured up to the required standard of assay. The pressure obtained was estimated to be about 75 to 150 pounds.

G. Mechanical Pressure Applied to the Drug. Bruns (58) used a process for percolation in which the drugs softened in the usual manner were compressed to the original volume of the dry drugs, then by constant and gradual reduction of the volume of the material by pressure, the percolation was effected. By this process about 0.33 of the amount of liquid ordinarily required was used.

Herzog and Fosse (59) made comparative experiments upon five drugs by percolation and the Bruns moistening method with use of the Hebel press and showed that although the latter gave satisfactory results in some cases it was, in its form at that time, not worthy of further consideration.

Kroeber (60) also compared the results of extraction of cascara sagrada, frangula, condurango and gentian by the Bruns method and by percolation. The Bruns method of successive pressing gave satisfactory results. The comparison was based on the density and the percent of solid extract, but no statements were found concerning the therapeutic efficiency of the products.

III. EXPERIMENTAL DATA ON BELLADONNA ROOT

1. Scope and General Methods - In order to note the effect of reduced pressure in the extraction of belladonna root several types of experiments were performed. The experiments can be divided into maceration and percolation. In some experiments the reduced pressure was applied to the dry belladonna root before adding the menstruum, and in other experiments the menstruum was added to the dry drug and reduced pressure

applied to the mixture. In every experiment a control was performed simultaneously with the reduced pressure experiment and in order to determine the effect of reduced pressure in extraction, the finished preparations were analyzed for total alkaloids and total extractive. In some instances various fractions of percolate were analyzed to note the progress of extraction at various stages.

2. Materials Used.

a. Drug - One hundred pounds of moderately coarsely powdered belladonna root was purchased from S. B. Penick and Company. The drug was thoroughly mixed to insure uniformity throughout the experimental work. The results of numerous assays of the drug according to the U.S.P. XI method gave an average of 0.464 per cent of the alkaloids of belladonna root. According to the U.S.P. XI moisture method for drugs containing no constituents volatile at 100°C. this shipment of belladonna root was found to contain 13.88 per cent of moisture.

b. Menstruum - The menstruum used throughout the experimental work was a mixture of 4 volumes of alcohol and 1 volume of distilled water as prescribed in the U.S.P. XI for making fluidextract of belladonna root.

c. Vacuum Pump - A Cenco Hyvac vacuum pump was used in all experiments to produce the reduced pressure.

d. Vacuum Gauge - A Bennert type vacuum gauge was used in taking readings of the reduced pressure.

3. Methods of Analysis.

a. Alkaloids - The regular U.S.P. XI assay for fluidextract of

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belladonna root was used. In some cases, as indicated, the weak percolate was assayed according to the U.S.P. XI assay for tincture of belladonna.

b. Total Extractive-Ten cubic centimeters of the percolate was placed in a tared beaker and the solvent evaporated on a water bath. The beaker containing the residue was placed in an electric oven at 105 degrees centigrade and dried until two successive weighings did not vary more than ten milligrams.

4. Tests of the Accuracy of the U.S.P. XI Assay of Fluidextract of Belladonna Root. In determining the accuracy of the U.S.P. XI assay of fluidextract of belladonna root it was thought best to use a known percentage solution of atropine in place of the fluidextract and determine the amount of atropine that could be obtained from the known solution.

A sample of atropine was secured. In determining the purity of the atropine the following test was performed: Four samples of atropine were accurately weighed and a definite amount of standard sulfuric acid added to each portion of weighed atropine. The excess acid was titrated with standard sodium hydroxide and the amount of atropine was calculated. The following results are given in Table I.

Table I

<u>Weight of Atropine</u>	<u>Cc. Sulfuric Acid 0.02283N</u>	<u>Cc. NaOH 0.02281N</u>	<u>Weight Atropine Shown By Titration</u>	<u>Per Cent Atropine Shown By Titration</u>
0.0840	20.00	7.40	0.08323	99
0.0918	20.00	6.22	0.09097	99
0.0804	20.00	7.90	0.07993	99
0.1083	20.00	3.80	0.10693	98

As shown above the amount of atropine shown by titration was 99 per cent of the weighed samples.

A chloroform solution of the atropine was made in a volumetric flask by first weighing accurately the amount of atropine used. The solution contained 0.4808 grams of atropine per 100 cc. of solution. This chloroform solution of atropine was assayed for total alkaloidal content by the process outlined in the U.S.P. XI for the assay of fluidextract of belladonna root. The results are given in Table II.

Table II

<u>Chloroform Solution Used in cc</u>	<u>Cc. Sulfuric Acid 0.02283N</u>	<u>Cc. NaOH 0.02281N</u>	<u>Weight Atropine Present</u>	<u>Weight Atropine Recovered</u>	<u>Per cent Atropine Recovered</u>
10.00	15.00	8.50	0.04808	0.04295	89.3
10.00	15.00	8.50	0.04808	0.04295	89.3
10.00	15.00	8.40	0.04808	0.04359	90.6
10.00	15.00	8.50	0.04808	0.04295	89.3
10.00	15.00	8.62	0.04808	0.04211	87.6

As shown by the results approximately 90 per cent of the atropine

was recovered. This indicated that the error in the process was about 10 per cent.

Another type of experiment was performed in which a definite amount of the atropine was weighed and dissolved in the U.S.P. XI menstruum for fluidextract of belladonna root and the mixture assayed. The results are given in Table III.

Table III

<u>Weight Atropine</u>	<u>Cc. Sulfuric Acid 0.02283N</u>	<u>Cc. NaOH 0.02281N</u>	<u>Weight Atropine Recovered</u>	<u>Per cent Atropine Recovered</u>
0.0455	20.00	13.78	0.04121	90.5
0.0802	20.00	8.78	0.07415	92.4
0.0608	20.00	11.28	0.05768	94.8
0.0922	20.00	6.70	0.08785	95.2

The results of the above experiment seem to indicate that in the U.S.P. XI process for the assay of fluidextract of belladonna root, 90 to 95 per cent of the alkaloids present is recovered.

Using the last two samples in the above table, it was determined what effect heat had on the official process. In one part of the process the substance is dissolved in chloroform, the chloroform evaporated on a water bath and heating continued for fifteen minutes. This procedure was repeated three times and it was determined that this heating reduced the results of the assay between 2 to 4 per cent.

5. Effect of Reduced Pressure on the Extraction of Belladonna Root by Maceration.

a. The Extraction of Ten Grams of Belladonna Root in Divided

Portions - In the present investigation in determining the effect of vacuum on the extraction of belladonna root, two types of vacuum experiments were performed. In the first experiment 2.50 Gm. of belladonna root was placed in each of four 50 cc. centrifuge tubes. Reduced pressure (pressure of 145 to 150 mm. of mercury) was applied for about two minutes and exactly 22.50 Gm. of menstruum was added from a burette. The reduced pressure was removed and the mixture allowed to stand fifteen minutes with stirring every five minutes. The tube plus drug and menstruum was centrifuged ten minutes, the liquid poured off and the tube and drug weighed to determine the amount of liquid imbibed. The macerates of the four tubes were combined and weighed. The total macerates were assayed for total extractive and alkaloidal content.

In the second experiment the same sized tubes and the same weight of drug and menstruum were used as in the first experiment. The menstruum was added under atmospheric pressure and the tubes were placed in a desiccator and reduced pressure of about 140 mm. of mercury was applied for fifteen minutes. The vacuum was removed every five minutes and the contents of the tubes stirred. The drug and macerates were treated as in the first experiment.

A control was also performed using the same amount of drug and menstruum. The control was treated as the two above experiments, except the vacuum was omitted. The two experiments and control were run in duplicate.

The amount of dry marc was calculated as follows: (Weight of drug

taken for extraction) minus (weight of moisture in drug) minus (weight of dissolved solids in filtrate) = (weight of dry marc). The weight of liquid imbibed by the marc was calculated as follows: (Weight of wet marc) minus (weight of dry marc) = (weight of liquid imbibed by the marc). The loss of menstruum during the process was determined by comparing the total weight of the materials used with the combined weight of the filtrate and wet marc.

The total alkaloidal content of the macerates was determined by the U.S.P. XI assay of tincture of belladonna, except that 25.00 Gm. of macerate was used in place of 100 cc. as specified by the U.S.P. XI. Each determination was performed in duplicate.

The results of the experiment are given in Table IV, A and B being the duplicates of each experiment.

Table IV

<u>First Experiment:-</u>	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	74.10	74.10	74.10
Amount of Liquid Imbibed	18.92	18.74	18.83
Weight of Dry Marc	7.017	7.064	7.041
Amount of Menstruum Lost	0.00	0.11	0.06
Total Extractive	1.595	1.548	1.572
Total Alkaloids	0.0359	0.0368	0.0364

(Table continued on next page)

<u>Second Experiment:-</u>	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	73.77	73.84	73.81
Amount of Liquid Imbibed	18.63	18.57	18.60
Weight of Dry Marc	7.023	7.061	7.042
Amount of Menstruum Lost	0.58	0.54	0.56
Total Extractive	1.589	1.551	1.570
Total Alkaloids	0.0368	0.0363	0.0366

Controls:-

Weight of Macerate	73.61	73.88	73.75
Amount of Liquid Imbibed	18.83	18.85	18.84
Weight of Dry Marc	7.015	7.023	7.019
Amount of Menstruum Lost	0.45	0.25	0.35
Total Extractive	1.597	1.589	1.593
Total Alkaloids	0.0368	0.0367	0.0367

From the above results it appears that reduced pressure does not increase the total alkaloidal content in extraction of belladonna root. Due to the fact that reduced pressure in the present investigation seemed to have no effect on the extraction of belladonna root for total alkaloids, it was thought advisable to extend the time of maceration under reduced pressure to about thirty minutes and observe the effect on the extraction.

b. The Extraction of Ten Gram Portions of Belladonna Root -

On continuing the present investigation in determining the effect of reduced pressure on the extraction of belladonna root, the two types of vacuum experiments, as performed in the 50-cc. centrifuge tubes, were somewhat modified. The entire 10.00 Gm. of belladonna root was placed in a wide mouth bottle of about 200 cc. capacity. A two-hole rubber stopper was placed in the mouth of the bottle. One of the holes was fitted with a glass tube connected to the vacuum pump. A separatory funnel was inserted in the other. A partial vacuum (pressure of about 130 to 135 mm. of mercury) was applied to the dry drug for thirty minutes. Ninety grams of menstruum was added to the dry drug under reduced pressure in rapid drops through the separatory funnel. Ten minutes were required for the adding of the menstruum. During the addition of the menstruum the vacuum remained constant. Air was allowed to enter the bottle and the mixture allowed to stand for thirty minutes with stirring every five minutes. The bottle containing the drug and menstruum was centrifuged ten minutes, in order to separate the drug from the menstruum, the liquid decanted and the bottle and drug weighed to determine the amount of liquid imbibed. The macerate was weighed and then assayed for total extractive and alkaloidal content.

In the second experiment performed using the same size of bottle, the same weight of drug and menstruum as above, the menstruum was added to the dry drug under atmospheric pressure. The bottle was then placed in a desiccator and a partial vacuum (pressure of 130 to 135 mm. of mercury) was applied for thirty minutes. Every five minutes air was

allowed to enter the bottle and the contents of the bottle thoroughly stirred. The drug and macerate were then treated as in the above experiment.

A control was also performed using the same amount of drug and menstruum but omitting the vacuum. The two experiments and control were run in duplicate.

The results of the experiments are given in Table V, A and B being the duplicates of each experiment.

Table V

<u>First Experiment:-</u>	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	73.77	73.34	73.56
Amount of Liquid Imbibed	18.86	19.23	19.05
Weight of Dry Marc	7.020	7.044	7.032
Amount of Menstruum Lost	0.36	0.39	0.38
Total Extractive	1.596	1.568	1.582
Total Alkaloids	0.0387	0.0387	0.0387

Control:-

Weight of Macerate	73.65	72.57	73.11
Amount of Liquid Imbibed	18.95	19.92	19.44
Weight of Dry Marc	7.012	7.044	7.026
Amount of Menstruum Lost	0.40	0.48	0.44
Total Extractive	1.600	1.568	1.584

(Table Continued on next page)

	<u>A</u>	<u>B</u>	<u>Averages</u>
Total Alkaloids	0.0383	0.0383	0.0383

Second Experiment:-

Weight of Macerate	72.92	72.26	72.59
Amount of Liquid Imbibed	19.29	19.67	19.58
Weight of Dry Marc	7.033	7.028	7.031
Amount of Menstruum Lost	0.77	0.75	0.76
Total Extractive	1.579	1.584	1.582
Total Alkaloids	0.0388	0.0381	0.0385

Control:-

Weight of Macerate	72.96	72.56	72.76
Amount of Liquid Imbibed	19.55	19.97	19.76
Weight of Dry Marc	7.017	7.008	7.013
Amount of Menstruum Lost	0.48	0.47	0.48
Total Extractive	1.595	1.604	1.599
Total Alkaloids	0.0385	0.0386	0.0386

From the above results it appears that vacuum does not increase the efficiency of the extraction of belladonna root. In all vacuum experiments performed so far, it was noticed that when the vacuum was broken, any drug particles that were floating immediately settled. In all cases where vacuum was used the drug settled or was compacted much better than in the controls where the vacuum was omitted.

c. The Extraction of Four Hundred Gram Portions of Belladonna Root - It was thought advisable to increase the amount of drug used in the vacuum experiments and observe the effect of vacuum upon the extraction on the larger amount of drug. In this experiment a higher vacuum was used. The vacuum was applied to the dry drug, the menstruum added and the vacuum broken and applied to the mixture several times. Four hundred grams of moderately coarsely powdered belladonna root was placed in a five liter round bottom flask. The flask was fitted with a two-hole rubber stopper in which was placed a separatory funnel and a glass tube for connection to the vacuum pump. The flask was then evacuated for thirty minutes to 14 mm. of mercury. Sixteen hundred grams of menstruum was gradually added through the separatory funnel to the drug, taking fifteen minutes for the addition of the menstruum. The mixture was shaken occasionally as the menstruum was being added. When all the menstruum had been added the vacuum gauge showed a pressure of 50 mm. of mercury. Air was then allowed to enter the flask and the contents of the flask were thoroughly shaken. The mixture was allowed to stand at atmospheric pressure for ten minutes with occasional shaking. A partial vacuum (pressure of about 65 mm. of mercury) was applied for ten minutes; the contents of the flask being shaken several times. The mixture was allowed to stand again for ten minutes at atmospheric pressure and then a pressure of 65 mm. of mercury was applied for an additional ten minutes. When a partial vacuum (pressure of 42 to 47 mm. of mercury) was applied to the mixture the macerate boiled,

however when the pressure was increased to 52 to 60 mm. of mercury the macerate did not boil. Therefore in the above instance where the vacuum was applied to the mixture of drug and menstruum a pressure of 65 mm. of mercury was applied to prevent boiling of the menstruum. The mixture was filtered through filter paper and the macerate weighed. The damp marc was also weighed in order to determine the amount of liquid imbibed. The macerate was assayed for total extractive and total alkaloidal content.

The above procedure was repeated and in each case a control was performed at the same time. No vacuum was used in the control, however the same size flask, the same amount of drug and menstruum and the same time of maceration were employed as in the vacuum experiment. Each time the mixture of drug and menstruum in the vacuum experiment was shaken, the mixture of drug and menstruum in the control was also shaken. The flask in which vacuum was applied was observed to be somewhat cooler than the control flask.

The results of the experiments are given in Table VI, A and B being the duplicates of each experiment.

Table VI

<u>Vacuum Experiment:-</u>	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	968	965	966.5
Amount of Liquid Imbibed	696.4	700.5	698.5
Weight of Dry Marc	307.1	305.0	306.1
Amount of Menstruum Lost	28.5	29.5	29.0

(Table continued on next page)

	<u>A</u>	<u>B</u>	<u>Averages</u>
Total Extractive	37.37	39.51	38.44
Total Alkaloids	1.092	1.174	1.133
<u>Control:-</u>			
Weight of Macerate	978	992	985
Amount of Liquid Imbibed	705.6	695.2	700.4
Weight of Dry Marc	306.4	306.3	306.4
Amount of Menstruum Lost	10.0	6.5	8.2
Total Extractive	38.09	38.16	38.12
Total Alkaloids	1.138	1.158	1.148

It appears that reduced pressure has no effect on the extraction of belladonna root. The results obtained in the vacuum experiment on the amount of liquid imbibed by the drug and the total alkaloidal content and total extractive of the macerate came within experimental error in comparison to the results obtained in the controls.

In performing the above experiment it was noticed that when vacuum was applied to the drug and menstruum, and also to an empty flask, the vacuum flask was much cooler than the control flask. This difference in temperature was due to the cooling caused by evaporation of the menstruum or to evaporation of moisture in the flask or expansion of the air in the flask.

d. The Extraction of Four Hundred Gram Portions of Belladonna Root at Constant Temperature - Due to the fact that in the previous

vacuum experiments it was noticed that the vacuum flask, when vacuum was applied, was much cooler than the control flask, it was thought advisable to work under constant temperature in both the vacuum and control flasks and notice the effect of vacuum on the extraction of belladonna root. The same flasks and the same amount of menstruum and drug as used in the previous experiment were used in this experiment. A thermometer was inserted through the stopper in each of the flasks in order that the temperature of each flask could be observed.

Four hundred grams of moderately coarsely powdered belladonna root was placed in the five liter round bottom flask. The flask containing the dry drug was then evacuated to 9 mm. of mercury for thirty minutes. The temperature in the vacuum flask, after thirty minutes of evacuation, was observed to be 17°C . while the temperature in the control flask was 21°C . Sixteen hundred grams of menstruum was added through the separatory funnel to the evacuated drug in the flask, taking fifteen minutes for the addition of the menstruum. The mixture was shaken occasionally as the menstruum was being added. When all the menstruum had been added the temperature of the vacuum flask was 18°C , and that of the control flask was 21°C . At this point the vacuum gauge showed a pressure of 37 mm. of mercury. The vacuum was then broken. The vacuum flask was then placed in a water bath at 30°C . and the temperature of the contents of the vacuum flask was regulated to the temperature of the control flask. The contents of both flasks were thoroughly shaken in order that the heat would be equally distributed and also in order to mix the contents. As soon as

the temperatures in the two flasks were equal, which took about eleven minutes after the vacuum was broken, the contents of each flask were filtered. The macerate and marc were treated as in the previous experiments. The above experiment was performed in duplicate.

The results of the experiments are given in Table VII, A and B being the duplicates of each experiment.

Table VII

<u>Vacuum Experiment:-</u>	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	1007	974	990.5
Amount of Liquid Imbibed	668.5	695.4	682.0
Weight of Dry Marc	306.1	309.6	307.9
Amount of Menstruum Lost	18.5	21.0	19.75
Total Extractive	38.43	34.95	36.69
Total Alkaloids	1.136	0.947	1.042

Control:-

Weight of Macerate	1043	1020	1031
Amount of Liquid Imbibed	648.0	670.6	659.3
Weight of Dry Marc	303.0	303.4	303.2
Amount of Menstruum Lost	6.0	6.0	6.0
Total Extractive	41.47	41.06	41.26
Total Alkaloids	1.153	1.138	1.146

In the above results it is noticed that the control extracted a little more total extractive and total alkaloids than the vacuum experiment.

It appears that the equilibrium between the menstruum and total extractive and total alkaloidal content in the vacuum and control experiments is reached within a few minutes after the menstruum and drug are mixed. It had been noticed in all previous vacuum experiments that the drug settled much better than in the controls. This may be an indication that the menstruum has entered the cells of the drug much better than under atmospheric pressure, although in some of the experiments the proportion of liquid imbibed was the same.

6. Effect of Reduced Pressure on the Extraction of Belladonna Root by Percolation.

Two hundred and fifty grams of moderately coarsely powdered belladonna root was placed in a pyrex glass tube 64 cm. long and 4 cm. in internal diameter. The drug in the dry state was packed from the top. In this method the drug was introduced into the percolator in small portions with slight agitation of the percolator to promote even distribution, and after all the drug had been thus introduced it was packed from the top, using a long wooden rod with a cork attached to the end and starting with light pressure which was gradually increased. Partial vacuum (9 mm. pressure of mercury) was applied through the receiving flask for thirty minutes. The menstruum was then added to the drug under reduced pressure. The vacuum remained constant throughout the addition of the menstruum.

When the menstruum reached the lower orifice of the percolator air was allowed to enter the percolator and the mixture allowed to macerate for twenty-four hours at atmospheric pressure. The percolation was then carried out collecting the percolate in two 125 cc. portions. No vacuum was used when the drug was percolated.

The above experiment was conducted simultaneously in duplicate. That is, the two pyrex glass tubes in which the vacuum experiments were performed, were connected to the same vacuum tube, thus evacuating the two pyrex tubes at the same time. Two controls, no vacuum being used, were run simultaneously with the vacuum experiments.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table VIII.

Table VIII

A. Experimental Details

	I	<u>Vacuum</u> II	III	<u>Controls</u> IV
Volume of Packed Drug in cc.	505	505	505	505
Height of Drug Column in cm.	37.0	38.0	38.0	37.0
Rate which Menstruum was Added in cc. per minute	1.7	1.64	0.21	0.21
Time Required for Liquid to Reach Lower Orifice in minutes	205	213	1415	1415

(Table continued on next page)

	<u>Vacuum</u>		<u>Controls</u>	
	I	II	III	IV
Time of Maceration After Menstruum Reached Lower Orifice in Hours	24	24	24	24
Rate of Flow in cc. per Minute:				
First Fraction	0.59	0.60	0.26	0.23
Second Fraction	0.74	0.72	0.30	0.30
Menstruum Absorbed by Drug in cc.	350	350	300	300
Total Menstruum Used in cc.	610	610	590	585

B. Assay Results

Vacuum Experiment:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
<u>No. I</u>		
First Fraction - 125 cc.	1.021	20.74
Second Fraction- 125 cc.	0.059	14.70
Total	<u>1.080</u>	<u>35.44</u>
<u>No. II</u>		
First Fraction - 125 cc.	1.012	20.43
Second Fraction- 125 cc.	0.060	14.99
Total	<u>1.072</u>	<u>35.42</u>

Controls:-

<u>No. III</u>		
First Fraction - 125 cc.	1.120	22.02
Second Fraction- 125 cc.	0.000	13.55
Total	<u>1.120</u>	<u>35.57</u>
<u>No. IV</u>		
First Fraction - 125 cc.	1.157	21.67
Second Fraction- 125 cc.	0.000	16.06
Total	<u>1.157</u>	<u>37.73</u>

As shown by the results in the above experiments it appears that reduced pressure has no beneficial effect upon the extraction of moderately coarsely powdered belladonna root. In the controls more total alkaloidal extractive and total extractive were extracted than in the vacuum experiments. In the controls it took a much longer time for the liquid to reach the lower orifice than in the vacuum experiments, therefore allowing a longer time for the liquid to dissolve the plant constituents. In the above experiments the only advantage that was observed for the vacuum was that the extraction of the belladonna root was completed in a much shorter time than in the controls.

It is interesting to note that in the above experiments the first 250 cc. of percolate from 250 Gm. of drug comes within the assay requirements for fluidextract of belladonna root.

The previous experiment was repeated using the same amounts of drug and same conditions of reduced pressure, etc. However in this experiment the percolates in the experiments using reduced pressure were collected at the same rate as the corresponding percolates in the controls. The difference in the rate of flow of the corresponding percolates in the preceding experiment was thought to be a possible cause of the difference in the results of the reduced pressure part of the experiment as compared to the controls. After collecting the first fraction in each of the four tubes in the present experiment, the drug was allowed to macerate fourteen and one-half hours before collecting the second fraction.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table IX.

Table IX

A. Experimental Details

	<u>Vacuum</u>		<u>Controls</u>	
	I	II	III	IV
Volume of Packed Drug in cc.	505	505	505	505
Height of Drug Column in cm.	37.0	38.0	38.0	37.0
Rate which Menstruum was Added in cc. per Minute	3.0	2.6	0.21	0.22
Time Required for Liquid to Reach Lower Orifice	115	135	1455	1380
Time of maceration After Menstruum Reached Lower Orifice in Hours	20.7	20.5	20.7	22
Rate of Flow in cc. per Minute:				
First Fraction	0.21	0.21	0.21	0.21
Second Fraction	0.29	0.29	0.28	0.29
Menstruum Absorbed by Drug in cc.	350	350	300	300
Total Menstruum Used in cc.	603	604	565	569

(Table continued on next page)

B. Assay Results

Vacuum Experiment:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
No. I		
First Fraction - 125 cc.	1.071	20.48
Second Fraction - 125 cc.	0.076	16.46
Total	<u>1.147</u>	<u>36.94</u>

No. II		
First Fraction - 125 cc.	1.083	19.96
Second Fraction - 125 cc.	0.076	17.20
Total	<u>1.159</u>	<u>37.16</u>

Control:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
No. III		
First Fraction - 125 cc.	1.114	20.05
Second Fraction - 125 cc.	0.000	15.19
Total	<u>1.114</u>	<u>35.24</u>

No. IV		
First Fraction - 125 cc.	1.123	19.11
Second Fraction - 125 cc.	0.000	16.23
Total	<u>1.123</u>	<u>35.34</u>

In the following table averages are given of the analyses for alkaloids and extractive matter of the preceding two experiments:-

Vacuum Experiment:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
Average	1.115	36.24

Control:-

Average	1.129	35.97
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As shown by the averages of the experiments reduced pressure has

no appreciable effect on the extraction of moderately coarsely powdered belladonna root. It seems that the only advantage of reduced pressure in the extraction of the belladonna root was that the menstruum penetrated the entire columns of drug much more quickly when reduced pressure was used than in the controls. This may be an advantage of reduced pressure in the percolation of those drugs in which the menstruum will not penetrate the packed drug in the percolator under atmospheric pressure. In the controls of the preceding two experiments the second fraction (125 cc.) of percolate from 250 Gm. of drug was free from alkaloids, however, in the vacuum experiments the second fraction still contained alkaloids. This indicates that the extraction of alkaloids is more rapid in the controls than in the vacuum experiments. In the controls the menstruum penetrated the column of packed drug much more slowly than in those experiments performed under reduced pressure. It seems that this difference in time that it takes the menstruum to reach the lower orifice of the percolator gives the menstruum in the controls more time to dissolve the plant constituents.

7. Percolation of Belladonna Root at Atmospheric Pressure. It was noticed that in the percolation of belladonna root in the pyrex glass tubes in the previous experiment practically all the total alkaloids were extracted in the first fraction of the percolate. Each cubic centimeter of this first fraction represented two grams of the belladonna root. In using 250 Gm. of belladonna root, practically all the alkaloids were extracted in the first 125 cc. of the percolate. In

some cases all the alkaloids were extracted in this first fraction.

Inasmuch as such an efficient extraction occurred in the tubes of the previous experiment, it was thought advisable to see if such an efficient extraction of belladonna root could be obtained in an ordinary percolator. For the present experiment the dimensions of the percolators were as follows: length 56.5 cm., internal diameter at the top 11.3 cm. This experiment was divided into two parts. In one part the dry moderately coarsely powdered belladonna root was packed in the percolator and in the other part of the experiment the drug was first moistened with 25 cc. of menstruum per 100 Gm. of drug. After the drug had been moistened the mixture was allowed to stand about fifteen minutes before being packed in the percolator. In both cases the method of packing the drug from the top was used. In this method the drug was introduced into the percolator in small portions with slight agitation of the percolator to promote even distribution, and after all the drug had been thus introduced it was packed from the top, using a wooden potato masher and starting with light pressure which was gradually increased.

In each part of the experiment 1200 Gm. of moderately coarsely powdered belladonna root was packed in the percolator as described above. The menstruum was added to the drug by inverting a round-bottomed flask above the percolator. The round-bottomed flask contained a rubber stopper in which a glass tube was inserted. When the flask was placed in position, the glass tube extended to within about one inch of the surface of the drug in the percolator. As the

menstruum ran through the drug, more menstruum was automatically added thus keeping an excess of about one inch of menstruum above the drug. When the menstruum reached the lower orifice of the percolator, the lower orifice was closed and the drug allowed to macerate twelve hours. Percolation was then started and the percolate of each percolator was collected in three successive 600 cc. fractions. The drug was allowed to macerate fourteen and one-half hours between the collection of each fraction. Conditions such as time of maceration and rate of flow were kept constant throughout both types of experiments. The two types of experiments were performed in duplicate and in order to check on extraction each fraction was analyzed for total alkaloidal content and total extractive.

A summary of the details of the process and an average of the analyses of the various fractions for alkaloids and extractive matter are shown in Table X.

Table X

A. Experimental Details

	<u>Drug Packed Dry in Percolator</u>		<u>Drug Moistened Then Packed in Percolator</u>	
	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>
Volume of Packed Drug in cc.	2450	2225	3225	3050
Height of Drug Column in cm.	34.0	31.0	42.0	39.8
Time Required for Liquid to Reach Lower Orifice in Minutes	780	930	285	360

(Table continued on next page)

	Drug Packed Dry in Percolator		Drug Moistened Then Packed in Percolator	
	I	II	III	IV
Time of Maceration After Menstruum Reached Lower Orifice in Hours	12	12	13	13
Rate of Flow in cc. Per Minute:				
First Fraction	1.0	0.6	1.0	0.6
Second Fraction	1.0	1.0	1.0	1.0
Third Fraction	1.0	1.0	1.0	1.0
Menstruum Absorbed by Drug in cc.	1450	1280	1705	1500
Total Menstruum Used in cc.	3420	3235	3675	3600

B. Assay Results

Drug Packed Dry:-

	Total Alkaloidal Extractive in Gms.	Total Extractive in Gms.
<u>No. I</u>		
First Fraction - 600 cc.	5.081	87.32
Second Fraction - 600 cc.	0.182	66.13
Third Fraction - 600 cc.	0.034	57.71
Total	<u>5.297</u>	<u>211.16</u>
<u>No. II</u>		
First Fraction - 600 cc.	5.181	77.72
Second Fraction - 600 cc.	0.169	54.23
Third Fraction - 600 cc.	0.144	58.86
Total	<u>5.494</u>	<u>190.81</u>

Drug Moistened Then Packed:-

<u>No. III</u>		
First Fraction - 600 cc.	5.136	79.19
Second Fraction - 600 cc.	0.238	82.99
Third Fraction - 600 cc.	0.023	53.47
Total	<u>5.397</u>	<u>215.65</u>

(Table continued on next page)

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
No. IV		
First Fraction - 600 cc.	4.949	82.72
Second Fraction - 600 cc.	0.447	91.36
Third Fraction - 600 cc.	0.067	53.12
Total	<u>5.463</u>	<u>227.20</u>

The following table shows the averages of the two experiments performed on percolating belladonna root when packed dry and when packed after moistening the drug with 25 cc. of menstruum per 100 Gm. of drug. The figures in the table represent the total alkaloids and total extractive matter in 1800 cc. of percolate from 1200 Gm. of belladonna root.

Drug Packed Dry:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
Average	5.40	201.0

Drug Moistened Then Packed:-

Average	5.44	221.4
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In percolating moderately coarsely powdered belladonna root in an ordinary percolator the rate of extraction of total alkaloids and extractive matter was not as rapid as when the belladonna was percolated in the long tubes. In some of the experiments performed in the tubes the second fraction of percolate (125 cc.) from 250 Gm. of drug was entirely free of alkaloids. In every experiment performed on percolating belladonna in an ordinary percolator there were some alkaloids present in the third fraction (600 cc.) from 1200 Gm. of the drug. However the results obtained from percolating belladonna

in an ordinary percolator indicate that the first 1200 cc. of percolate from 1200 Gm. of drug meets the requirement of the U.S.P XI fluidextract of belladonna root in alkaloidal content.

The total amount of alkaloids extracted was practically the same regardless of whether the drug was packed dry or moistened before packing. However the percolates from the previously moistened drug contained more total extractive matter.

IV. EXPERIMENTAL DATA ON CINCHONA

1. Scope and General Methods - Cinchona contains a higher percentage of alkaloids than belladonna root and for this reason cinchona was chosen as the next drug to extract under reduced pressure. If reduced pressure has any beneficial effect on the extraction of drugs it seems that it should be noticed in the extraction of belladonna, which contains a small percentage of alkaloids, or in cinchona, which contains a high percentage of alkaloids. The experiments performed on cinchona were maceration and percolation under reduced pressure. In every experiment a control was performed simultaneously with the reduced pressure experiment and in order to determine the effect of reduced pressure in extraction the finished preparations were analyzed for total alkaloids and total extractive. In some instances various fractions of percolate were analyzed to note the progress of extraction at various stages.

2. Materials Used.

- a. Drug - Fifty pounds of moderately coarsely powdered yellow

cinchona was purchased from S. B. Penick and Company. The drug was thoroughly mixed to insure uniformity throughout the experimental work. The results of numerous assays of the drug according to the U.S.P. XI method gave an average of 8.26 per cent of the alkaloids of cinchona. According to the U.S.P. XI moisture method for drugs containing no constituents volatile at 100°C. this shipment of yellow cinchona was found to contain 8.95 per cent of moisture.

b. Menstruum - The menstruum used throughout the experimental work was a mixture of 100 cc. of glycerin, 100 cc. of diluted hydrochloric acid, and 800 cc. of alcohol as Menstruum I, and a mixture of 4 volumes of alcohol and 1 volume of distilled water as Menstruum II as prescribed in the U.S.P. X for making fluidextract of cinchona.

c. Vacuum Pump - A Cenco Hyvac vacuum pump was used in all experiments to produce the reduced pressure.

d. Vacuum Gauge - A Bennert type vacuum gauge was used in taking readings of the reduced pressure.

3. Method of Analysis.

a. Alkaloids - The regular U.S.P. X assay for fluidextract of cinchona was used. Asbestos fiber was used as the absorbent.

b. Total Extractive - Ten cubic centimeters of the percolate was placed in a tared beaker and the solvent evaporated on a water bath. The beaker containing the residue was placed in an electric oven at 105 degrees centigrade and dried until the difference between two successive weighings was not over ten milligrams.

4. Effect of Reduced Pressure on the Extraction of Yellow Cinchona by Percolation - Three hundred grams of moderately coarsely powdered yellow cinchona was placed in a pyrex glass tube 64 cm. long and 4 cm. in internal diameter. The drug in the dry state was packed from the top. In this method the drug was introduced into the percolator in small portions with slight agitation of the percolator to promote even distribution, and after all the drug had been thus introduced it was packed from the top, using a long wooden rod with a cork attached to the end and starting with light pressure which was gradually increased. The drug was packed until the column of drug was 40.5 to 41 cm. in height. As observed in previous experiments, it took the menstruum much longer to reach the lower orifice in the controls, in which no reduced pressure was used, than in the tubes in which reduced pressure was used. Hence it was thought advisable to first add the menstruum to the controls and, when the menstruum had almost reached the lower orifice, then start adding the menstruum to the drug under reduced pressure. This procedure was followed and it was found that the drug was packed too tight for the menstruum to penetrate the column of drug at atmospheric pressure. After standing two days the drug in the control tubes had absorbed only twenty to thirty cubic centimeters of menstruum. These controls were discarded and fresh dry yellow cinchona was placed in the tubes without forcibly packing the drug. However the drug was allowed to remain tightly packed in the tubes to which reduced pressure was applied. Reduced pressure (pressure of 8 mm. of mercury) was

applied through the receiving flask for thirty minutes. The menstruum was then added to the drug under reduced pressure. The reduced pressure remained constant throughout the addition of the menstruum. When the menstruum reached the lower orifice of the percolator air was allowed to enter the percolator and the mixture allowed to macerate for seventeen hours at atmospheric pressure. The percolation was then carried out collecting the percolate in three successive 150 cc. portions. Vacuum was not used when the drug was percolated. The control was run simultaneously with the above percolation.

The above experiments were conducted in duplicate. A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive are shown in Table XI.

Table XI

A. Experimental Details

	I	<u>Vacuum</u>	II	III	<u>Controls</u>	IV
Volume of Packed Drug in cc.	540		530	675		650
Height of Drug Column in cc.	41.0		40.5	50.5		48.5
Rate at which Menstruum was added in cc per Minute	1.80		1.16	0.24		0.21
Time Required for Liquid to Reach Lower Orifice in Minutes	199		310	1755		1965

(Table continued on next page)

	<u>Vacuum</u>		<u>Controls</u>	
	I	II	III	IV
Time of Maceration After Menstruum Reached Lower Orifice in Hours	17	17	17	17
Rate of Flow in cc. per Minute				
First Fraction	0.11	0.07	0.26	0.17
Second Fraction	0.19	0.10	0.48	0.27
Third Fraction	0.28	0.17	0.51	0.36
Menstruum Absorbed by Drug in cc.	360	360	420	410
Total Menstruum used in cc.	865	868	1010	980

B. Assay Results

Vacuum Experiments:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
<u>No. I</u>		
First Fraction - 150 cc.	7.37	48.43
Second Fraction - 150 cc.	5.33	32.65
Third Fraction - 150 cc.	3.16	18.08
Total	15.86	99.16
<u>No. II</u>		
First Fraction - 150 cc.	7.34	47.12
Second Fraction - 150 cc.	5.32	34.91
Third Fraction - 150 cc.	3.53	20.60
Total	16.19	102.63

Control:-

<u>No. III</u>		
First Fraction - 150 cc.	7.27	47.30
Second Fraction - 150 cc.	4.84	31.85
Third Fraction - 150 cc.	3.23	18.24
Total	15.34	97.39

(Table continued on next page)

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
No. IV		
First Fraction - 150 cc.	7.14	45.19
Second Fraction - 150 cc.	6.05	36.66
Third Fraction - 150 cc.	2.98	16.50
Total	16.17	98.35

In the following table averages are given of the analyses for alkaloids and extractive matter of the two preceding experiments.

Vacuum Experiment:-

	<u>Total Alkaloidal Extractive in Gms.</u>	<u>Total Extractive in Gms.</u>
Average	16.03	100.90

Control:-

Average	15.76	97.87
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In the experiments performed under reduced pressure greater quantities of alkaloids and total extractive matter were extracted than in the controls, but the differences are thought to be within the range of experimental error.

5. Effect of Reduced Pressure on the Extraction of Cinchona by

Maceration - In the percolation of drugs under reduced pressure many factors, such as the rate at which the menstruum is added to the drug and the rate at which the percolate is collected, must be considered. In maceration with an excess of menstruum such factors as mentioned above are eliminated, hence more definite conclusions can be drawn as to the effect of reduced pressure on the extraction.

Four hundred grams of moderately coarsely powdered yellow cinchona was placed in a five-liter round-bottomed flask. The flask

was fitted with a two-holed rubber stopper in which was placed a separatory funnel and a glass tube for connection to the vacuum pump. The flask was then evacuated for thirty minutes to a pressure of 12 mm. of mercury. Sixteen hundred grams of Menstruum I was gradually added through the separatory funnel to the evacuated drug, taking fifteen minutes for the addition of the menstruum. The mixture was shaken occasionally as the menstruum was being added. When all the menstruum had been added the vacuum gauge showed a pressure of 45 mm. of mercury. Air was then allowed to enter the flask and the contents of the flask were thoroughly shaken. The mixture was allowed to stand at atmospheric pressure for ten minutes with occasional shaking. A partial vacuum (pressure of 45 to 50 mm. of mercury) was applied for ten minutes, the contents of the flask being shaken several times. The mixture was allowed to stand again for ten minutes at atmospheric pressure and then a partial vacuum (pressure of 45 to 50 mm. of mercury) was applied for an additional ten minutes. When a partial vacuum (pressure of about 45 mm. of mercury) was applied to the mixture the macerate boiled, however, when the pressure was increased to about 50 mm. of mercury the macerate did not boil. Therefore in the above instance where the vacuum was applied a pressure of about 50 mm. of mercury was applied to prevent boiling of the menstruum. The mixture was filtered through filter paper and the macerate weighed. The damp marc was also weighed in order to determine the amount of liquid imbibed. The macerate was assayed for total extractive and total alkaloidal content.

The amount of dry marc was calculated as follows: (Weight of drug taken for extraction) minus (weight of moisture in drug) minus (weight of dissolved solids in filtrate) = (weight of dry marc). The weight of liquid imbibed by the marc was calculated as follows: (Weight of wet marc) minus (weight of dry marc) = (weight of liquid imbibed by the marc). The loss of menstruum during the process was determined by comparing the total weight of the material used with the combined weight of the filtrate and wet marc.

The above procedure was repeated and in each case a control was performed at the same time. No vacuum was used in the control, however the same size of flask, the same amount of drug and menstruum and the same time of maceration were employed as in the vacuum experiment. Each time the mixture of drug and menstruum in the vacuum experiment was shaken, the mixture of drug and menstruum in the control was also shaken.

The results of the experiments are given in Table XII, A and B being the duplicates of each experiment.

Table XII

Vacuum Experiment:-

	<u>A</u>	<u>B</u>	<u>Averages</u>
Weight of Macerate	881.5 (960 cc.)	928 (1011 cc.)	904.8 (985.5 cc.)
Amount of Liquid Imbibed	883.8	829.1	856.5
Weight of Dry Marc	188.2	187.9	188.1

(Table continued on next page)

	<u>A</u>	<u>B</u>	<u>Averages</u>
Amount of Menstruum Lost	36	37	36.5
Total Extractive	176.0	176.3	176.2
Total Alkaloids	14.36	15.06	14.71

Control:-

Weight of Macerate	926.5 (1015 cc.)	973 (1062 cc.)	949.8 (1038.5 cc.)
Amount of Liquid Imbibed	869.9	832.0	850.9
Weight of Dry Marc	188.6	178.0	183.3
Amount of Menstruum Lost	0	1	0.5
Total Extractive	175.6	186.2	180.9
Total Alkaloids	13.74	15.22	14.48

It appears that reduced pressure has no beneficial effect on the extraction of yellow cinchona. The results obtained in the reduced pressure experiments and in the controls differ slightly, however, these differences are within the experimental error.

V. GENERAL DISCUSSION OF RESULTS

Many authors have stated that reduced pressure is beneficial in the extraction of drugs. However very little experimental work is available in which products obtained by use of reduced pressure were compared with preparations made without use of vacuum.

F. Chilson (51) stated that the rate of solution is influenced by mixing and heat, but that it has not been shown that vacuum or pressure

will alter solution rate to an appreciable degree. In the present investigation a control was performed with each reduced pressure experiment and the two products obtained were compared as to total alkaloids and total extractive matter.

In analyzing the various percolates of belladonna root in the present investigation the regular U.S.P. XI method for assaying fluidextract of belladonna root was used. Several experiments were performed in order to check the accuracy of the U.S.P. XI assay of fluidextract of belladonna root. The results indicated that 90 to 95 per cent of the alkaloids were recovered. This error in the method of assay was equalized in the present investigation by performing a control with each reduced pressure experiment. Both the macerates or percolates obtained in the reduced pressure experiment and control were analyzed simultaneously, therefore a direct comparison could be made between the two different experiments.

1. Advantages of Vacuum Maceration . S. P. Duffield (35) stated that when a ground drug was evacuated the drug particles gave up the air enclosed in them and when the menstruum was allowed to enter, it was forced into the pores by pressure of the air outside. Duffield said a more perfect maceration could be obtained by this method than by any other method previously adopted. R. F. Fairthorne (36) stated that the advantage of his apparatus was that a vacuum was produced in each drug cell which was at once filled up upon contact with the liquid. W. C. Peck (52) stated that the application of vacuum has two effects: (a) it withdraws all air from the finely divided drug so that

the menstruum has a free entry; (b) on the vacuum being broken, the pressure of the atmosphere forces the menstruum into the drug.

E. Kessler (61) concluded that extraction of drugs in the absence of air resulted in more complete extraction since the solvent was not prevented from entering the drug cell by the air which is normally present there.

2. Disadvantages of Vacuum Maceration - H. Breddin (62) did not agree with E. Kessler (61) concerning the influence of air in the drug on the process of extraction. Breddin stated that he could not see any value in removing the air. He stated that if such a process does actually have the effect of introducing more solvent, this would have a diluting effect on the tincture so prepared.

3. Vacuum Maceration - In the present investigation the results show that the quantity of menstruum imbibed by the drug is not increased by reduced pressure. It seemed to be the opinion of S. P. Duffield (35), R. F. Fairthorne (36), E. Kessler (61) and W. C. Peck (52) that when comminuted drugs were evacuated they gave up the air enclosed in the drug cells and when the menstruum was added it was forced into the pores by pressure of the air outside. If this should be the case it seems that the amount of liquid imbibed in the drug under reduced pressure would be greater than the amount of liquid imbibed by an equal quantity of drug when extracted at atmospheric pressure. However, our results show that moderately coarsely powdered belladonna root and moderately coarsely powdered cinchona did not imbibe any more liquid when macerated under reduced pressure than when macerated at

atmospheric pressure. The statements of Duffield (35), Fairthorne (36), Kessler (61) and Peck (52) apparently were based on opinion rather than fact.

When belladonna root and yellow cinchona were extracted under reduced pressure, it was noticed that the drug settled much better than when extracted at atmospheric pressure. This, however, is not due to a greater imbibition of liquid but may be due to the removal of adsorbed air from the surface of the drug particles. It may be possible that drugs in larger pieces, such as chips and blocks, would imbibe a greater amount of liquid when treated with the menstruum under reduced pressure than when treated with the liquid at atmospheric pressure but such is not the case with the powdered drugs which were studied.

W. C. Peck (52) concluded from his experiments that vacuum maceration increased the total extractive in compound tincture of gentian and in preparations of vanilla beans obtained by maceration with an excess of liquid. He stated that he withdrew samples at intervals and assayed for total extractive and then compared the results with a control. If Peck withdrew equal samples from the reduced pressure experiment and control for the analyses of total extractive, it would be expected that the sample extracted under reduced pressure would contain a slightly higher concentration of total solids than the control. This is due to the fact that when vacuum is applied to a liquid it evaporates more rapidly and more of the liquid is lost, thus concentrating the extractive matter in the macerate. This error was eliminated in the present investigation by weighing the total macerate and

calculating the total solids for the entire macerate. In the present investigation reduced pressure did not increase the extraction of total extractive matter or total alkaloids.

4. Advantage of Percolation under Reduced Pressure - The advantages of percolation under reduced pressure were stated by J. G. Beard (41) to be (a) saving of menstruum and (b) economy of time. Good results were obtained in the use of the mulcolation process by Zimmerman (63). Fluidextract of condurango was made by the process of evacolation by E. Kessler (45) and he claimed a better fluidextract was produced than by the official simple percolation process. E. Kessler (64) claimed the following advantages of evacolation over the present official simple percolation and maceration methods for making fluid-extracts and tinctures; (a) fluidextracts were rapidly prepared at low cost, (b) the disadvantages of heat were avoided, (c) all contact of the preparation with metal was avoided, (d) the process was simple. On the basis of color, E. Kessler (61) claimed that fluid-extract of chamomile produced by this process was of good quality. Kessler stated that a tube 1 meter in length, previously evacuated, gave the same results as a tube 1.5 or 2 meters in length when no vacuum was used or even when pressure was used. W. Brandrup (65) extracted belladonna leaves by evacolation. Since 2.31 Gm. of the 2.60 Gm. of total alkaloids present in 500 Gm. of drug was obtained by evocolation, the author considered the process satisfactory. Working on several widely different types of drugs C. J. Blok and H. J. A. Ter Wee (48) prepared fluidextracts by evacolation. Based on the

active constituents present in the finished preparations, better fluidextracts were claimed to be made by evacuation than by diacollection or by percolation. By a slight variation of the evacuation process in the use of previously moistened and macerated cinchona instead of the dry drug, evacuation was used by J. Büchi and K. Feinstein (66). Analyses of the fluidextract for alkaloids and extractive matter showed the preparation was inferior to the fluidextract of cinchona made by simple percolation. By displacing the drug with regular menstruum instead of water, and by using cinchona in the form of a coarser powder, a fluidextract was produced that contained as much alkaloids and extractive matter as simple percolation and more than fluidextracts made by either diacollection or repercolation.

5. Disadvantages of Percolation under Reduced Pressure - H. Breddin (67) claimed the disadvantages of mulcolation were as follows: (a) the suction applied in mulcolation damaged the extraction, (b) the mulcolation apparatus could not be used to make small quantities of fluidextracts, (c) the mulcolation principle overlooked the fact that the extraction of some drugs required more tubes or a longer column than the extraction of others.

6. Vacuum Maceration in Percolator Followed by Ordinary Percolation - In the present investigation maceration under reduced pressure in the percolator followed by ordinary percolation did not increase the extraction of total alkaloids or total extractive. The only advantage of reduced pressure in the extraction of belladonna root and cinchona in the present experiments was that the menstruum penetrated the column of

drug much more quickly under reduced pressure than at atmospheric pressure and the rate of flow of percolate was more rapid in the reduced pressure experiments. The disadvantage of extraction under reduced pressure was found in the present investigation to be the loss of menstruum. On large scale extraction this may be a decided factor. The present results are in accord with the fact that patented large vacuum percolators have largely been dropped out of use. The results are also in accord with the conclusions of W. J. Husa and S. B. Yates (47) who found that when belladonna root in No. 40 powder was macerated under reduced pressure with 60 cc. of menstruum per 100 Gm. of drug before packing in a percolator, then appeared to be no particular advantage.

7. Ordinary Percolation - In the present investigation it was found that in using a pyrex glass tube 64 cm. long and 4 cm. in internal diameter for the extraction of belladonna root the first 250 cc. of percolate from 250 Gm. of drug met the requirements for U.S.P. XI fluidextract of belladonna root in total alkaloids. In some cases the first 125 cc. of percolate from 250 Gm. of drug met the requirements of the U.S.P. XI fluidextract of belladonna root even after the fraction had been diluted with an equal volume of the U.S.P. menstruum for preparing the official fluidextract. In using 250 Gm. of belladonna root, practically all and in some cases all the alkaloids were extracted in the first 125 cc. of the percolate.

Inasmuch as such an efficient extraction occurred in the pyrex tubes, it was thought advisable to see if such an efficient extraction

of belladonna root could be obtained in an ordinary percolator. Percolators 56.5 cm. in length and 11.3 cm. in internal diameter were selected and 1200 Gm. of belladonna root was extracted by percolation. In one case the drug was packed dry and in another case the drug was first moistened with 25 cc. of menstruum per 100 Gm. of drug before packing. In percolating belladonna root in an ordinary percolator the rate of extraction of total alkaloids and extractive matter was not as rapid as when belladonna root was percolated in the long tubes. However the results obtained from percolating belladonna root in an ordinary percolator indicated that the first 1200 cc. of percolate from 1200 Gm. of drug met the requirements of the U.S.P. XI fluidextract of belladonna root in alkaloidal content. The total amount of alkaloids extracted was practically the same regardless of whether the drug was packed dry or moistened with 25 cc. of menstruum per 100 Gm. of drug before packing. This is in accord with the results of W. J. Husa and S. B. Yates (47) who found that the rate of extraction of alkaloid was equally rapid when no liquid was used for moistening and when 25 cc. was used for 100 Gm. of drug.

VI. SUMMARY

The literature was reviewed concerning the effect of pressure and reduced pressure in drug extraction. The various types of extraction apparatus using pressure or reduced pressure were described and listed as to the year they were introduced and the manner of applying the pressure or reduced pressure.

Several experiments were performed on the U.S.P. XI assay of fluidextract of belladonna root in order to determine the accuracy of the method. It seems that the process is accurate within 5 to 10 per cent. This error in the method of assay was equalized in the present investigation by performing a control with each reduced pressure experiment. Both the macerates or percolates obtained in the reduced pressure experiment and control were analyzed simultaneously, therefore a direct comparison could be made between the two different experiments.

In order to determine the effect of reduced pressure on the extraction of moderately coarsely powdered belladonna root and moderately coarsely powdered yellow cinchona two general types of experiments were performed, namely, maceration and percolation. In the maceration process the dry drug was evacuated from two to thirty minutes and the menstruum added to the dry evacuated drug. In the percolation process the drug was packed dry in the percolator which was then evacuated and the menstruum added to the evacuated drug. In every experiment a control was performed which was identical with the reduced pressure

experiments in all respects with the exception that reduced pressure was not applied in the control.

In each experiment the macerate or percolate was analyzed for total alkaloids and total extractive, and in the maceration experiments, for the amount of liquid imbibed.

Reduced pressure did not increase the extraction of total alkaloids or total extractive matter in belladonna root or yellow cinchona, nor did reduced pressure increase the amount of menstruum imbibed by the drug.

In the present investigation the advantage of reduced pressure in the extraction of drugs was ascertained to be an aid in the penetration of the drug by the menstruum. In some cases, such as when cinchona was packed in the percolator, the menstruum was unable to flow through the column of drug. By the use of reduced pressure, in the case of cinchona, it was possible for the menstruum to penetrate the column of drug. Therefore it seems that by the use of reduced pressure it is possible for menstruum to penetrate columns of drug through which the menstruum could not flow at atmospheric pressure.

From a study of the extraction of belladonna root in a glass tube the results indicate that a full strength fluidextract of belladonna root can be made by slow percolation through a comparatively short column of drug without resorting to fractional collection of weak percolate from several portions of drug as in repercolation, and without use of heat to concentrate weak percolate as in U.S.P. simple percolation.

Belladonna root was also percolated in an ordinary percolator and the results indicate that a full strength fluidextract of belladonna root can be made without the collection of various fractions and without evaporating weak percolate. The results obtained in the ordinary percolator were not as efficient as the results obtained in the glass tube as a larger quantity of the active principles was present in the second half of the percolate.

Belladonna root was percolated in the ordinary percolator by packing the drug in the dry state and by first moistening the drug before packing with 25 cc. of menstruum per 100 Gm. of drug. The total amount of alkaloids extracted was practically the same regardless of whether the drug was packed dry or moistened before packing. This confirms the work of Huse and Yates (47) in which they found that the total amount of alkaloids extracted was practically the same regardless of whether the drug was packed dry or moistened with 25 cc. of menstruum per 100 Gm. of drug before packing.

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VIII. BIOGRAPHICAL ITEMS

George Robert Jones was born May 7, 1911, at Dunnellon, Florida. His undergraduate studies were pursued at the University of Florida. He holds the following degrees from the University of Florida: Graduate in Pharmacy (1933), Bachelor of Science in Pharmacy (1934) and Master of Science in Pharmacy (1936). He has been taking graduate work in Pharmacy at the University of Florida since 1934.

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This dissertation was prepared under the direction of the Chairman of the Candidate's Supervisory Committee, and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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